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ORIGINAL ARTICLE

Piperine Augments the Protective Effect of Curcumin Against Lipopolysaccharide-Induced Neurobehavioral and Neurochemical Deficits in Mice

Ashok Jangra,[1,5] Mohit Kwatra,[1] Tavleen Singh,[1] Rajat Pant,[1] Pawan Kushwah,[1] Yogita Sharma,[1] Babita Saroha,[2] Ashok Kumar Datusalia,[3] and Babul Kumar Bezbaruah[4]

Abstract—The aim of the present study was to investigate the protective effects of curcumin alone and in combination with piperine against lipopolysaccharide (LPS)-induced neurobehavioral and neurochemical deficits in the mice hippocampus. Mice were treated with curcumin (100, 200, and 400 mg/kg, p.o.) and piperine (20 mg/kg, p.o.) for 7 days followed by LPS (0.83 mg/kg, i.p.) administration. Animals exhibited anxiety and depressive-like phenotype after 3 and 24 h of LPS exposure, respectively. LPS administration increased the oxido-nitrosative stress as evident by elevated levels of malondialdehyde, nitrite, and depletion of glutathione level in the hippocampus. Furthermore, we found raised level of pro-inflammatory cytokines (IL-1β and TNF-α) in the hippocampus of LPS-treated mice. Pretreatment with curcumin alleviated LPS-induced neurobehavioral and neurochemical deficits. Furthermore, co-administration of curcumin with piperine significantly potentiated the neuroprotective effect of curcumin. These results demonstrate that piperine enhanced the neuroprotective effect of curcumin against LPS-induced neurobehavioral and neurochemical deficits.

KEY WORDS: depression; anxiety; curcumin; piperine; oxido-nitrostative stress.

INTRODUCTION

Depression is a debilitating, commonly occurring heterogeneous neuropsychiatric disorder which has a complex and multifactorial etiology. It is characterized by sadness, loss of interest or pleasure, feeling of guilt or low self-worth, disturbed sleep or appetite, feeling of tiredness, and poor concentration resulting in personal suffering and suicidal ideation, in addition to social and economic burden [1]. It has been evolved globally as the third most important cause of disability and visualized/computed to be the largest contributor to the burden of disease by the year 2030 [2]. Depression and anxiety are often found in a comorbid condition [3]. Presently, the drugs available for clinical use in the treatment of depression are deluged by delayed action, lower efficacy, side effects, and drug–drug interactions [4]. Moreover, depression symptoms are heterogeneous among patients that lead to inconsistent therapeutic responses of the current antidepressant drugs. Therefore, there is a need to search alternative pharmacological intervention to enhance current treatment outcomes which will be more effective with a few side effects.

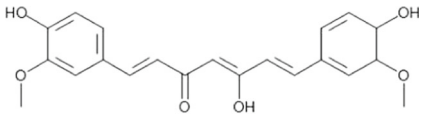
Immune dysregulation is a very common phenomenon found in major depression. Moreover, depressive symptoms have been found to be associated with autoimmune diseases at a high prevalence rate [5]. Several

[1] Department of Pharmacology and Toxicology, Laboratory of Neuroscience, National Institute of Pharmaceutical Education and Research, Guwahati, Assam 781032, India
[2] Department of Biotechnology, University Institute of Engineering & Technology (UIET), Maharishi Dayanand University, Rohtak, Haryana, India
[3] National Brain Research Centre, Manesar, Gurgaon, Haryana, India
[4] Department of Pharmacology, Gauhati Medical College, Guwahati, Assam, India
[5] To whom correspondence should be addressed at Department of Pharmacology and Toxicology, Laboratory of Neuroscience, National Institute of Pharmaceutical Education and Research, Guwahati, Assam 781032, India. E-mail: ashokjangra123@gmail.com

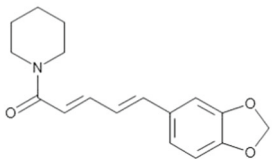
experiments had correlated the depression with various ranges of neurochemical disturbances within the brain such as increased level of pro-inflammatory cytokines, kynurenine pathway metabolites, acute phase protein, oxido-nitrosative stress, reduced neurogenesis, and mitochondrial dysfunction [6–10]. Neuroinflammation has been suggested to play a key role in several neurological diseases such as depression, Alzheimer's disease, epilepsy, Huntington's disease, multiple sclerosis, and Parkinson's disease [11, 12].

Peripheral administration of a single dose of the cytokine-inducer lipopolysaccharide (LPS) is a well-established model to study the behavioral and physiological responses in depression and anxiety [13]. LPS is a bacterial endotoxin that generates a dose-dependent activation of the innate immune response and exhibits both anxiety and depression-like behavior in mice by increasing the expression of pro-inflammatory mediators such as TNF-α, IFN-γ, IL-6, and IL-1β [1, 14, 15]. Furthermore, systemic administration of LPS causes oxido-nitrosative stress with altered antioxidant status [16]. Oxido-nitrosative stress and neuroinflammation lead to significant reduction in the neurotrophic factors like brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophin-3 (NT-3) levels in the brain [17].

Curcumin (Fig. 1a) is an active natural phytochemical (polyphenol) named as "Indian Saffron," extracted from the rhizome of *Curcuma longa* [18]. It has a long history as a traditional medicine in the Asian countries and has been using as the food additive, dietary spice, and herbal medicine [19]. It has been reported to possess various therapeutic properties such as an antioxidant, anti-inflammatory, hepato- and nephroprotective, anti-microbial, and neuroprotective properties [20–28]. It has also been reported to restore mitochondrial enzymes complex activity and thus weakens the release of reactive oxygen species [29]. In spite of numerous biological properties, curcumin has the limitation of having poor bioavailability. Attempts have been made to enhance the bioavailability of curcumin such as co-administration of curcumin with piperine (a bioavailability enhancer). Piperine (Fig. 1b) is a major alkaloidal constituent of *Piper nigrum* which inhibits the hepatic and intestinal glucuronidation and enhances the bioavailability of various drugs like curcumin, carbamazepine, and quercetin [18, 30, 31]. Thus, in the present study, we have investigated the possible neuroprotective effects of curcumin alone and in combination with piperine against LPS-induced various neurobehavioral and neurochemical deficits.



(a) (1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione



(b) 1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine

Fig. 1. Structure of curcumin (**a**) and piperine (**b**).

MATERIALS AND METHODS

Chemicals

LPS from *Escherichia coli*, serotype 0127:B8, curcumin, and piperine were purchased from Sigma-Aldrich, St. Louis, MO, USA. All other chemicals used were of standard analytical grade.

Animals

The experiments were performed in male Swiss albino mice (weight 22–25 g) from 0800 to 1400 hours and in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India guidelines. The study was approved by the Institutional Animal Ethics Committee (IAEC) (Approval no. MC/05/2015/41), Gauhati Medical College & Hospital (CPCSEA Registration no. 351, 3/1/2001). The animals were kept at room temperature (24 ± 1 °C) with 65 ± 10 % humidity, and 12 h light and dark cycles was maintained. Standard laboratory animal feed (Pranav Agro Industries Ltd. Pune, India) and water was provided *ad libitum*. Animals were acclimatized to the experimental conditions for a period of 1 week prior to the commencement of the experiment.

Preparation of Doses

Different doses of curcumin (100, 200, and 400 mg/kg) were selected based on the previous experimental study

[31]. Curcumin and piperine were prepared in peanut oil administered daily by oral route at the dose volume of 10 ml/kg. Lipopolysaccharide (0.83 mg/kg) serotype 0127: B8 was dissolved in endotoxin-free normal saline and administered intraperitoneally (i.p.) at the dose volume of 10 ml/kg.

Experimental Design

Mice were randomly divided into eight experimental groups each containing six animals. The experimental groups comprised of (Fig. 2):

Group I was treated with vehicle (peanut oil) of drugs for 7 days and then challenged with saline on the 7th day. This group served as the control group.

Group II was treated with vehicle (peanut oil) of curcumin for 7 days and then challenged with LPS (0.83 mg/kg, i.p.) on the 7th day. This group served as the LPS control group.

Group III was treated with curcumin (100 mg/kg, p.o.) for 7 days and then challenged with LPS (0.83 mg/kg, i.p.) on the 7th day.

Group IV was treated with curcumin (200 mg/kg, p.o.) for 7 days and then challenged with LPS (0.83 mg/kg, i.p.) on the 7th day.

Group V was treated with curcumin (400 mg/kg, p.o.) for 7 days and then challenged with LPS (0.83 mg/kg, i.p.) on the 7th day.

Group VI was treated with piperine (20 mg/kg, p.o.) for 7 days and then challenged with LPS (0.83 mg/kg, i.p.) on the 7th day

Group VII with treated with curcumin (100 mg/kg, p.o.) and piperine (20 mg/kg, p.o.) for 7 days and then challenged with LPS (0.83 mg/kg, i.p.) on the 7th day.

Group VIII with treated with curcumin (200 mg/kg, p.o.) and piperine (20 mg/kg, p.o.) for 7 days and then challenged with LPS (0.83 mg/kg, i.p.) on the 7th day.

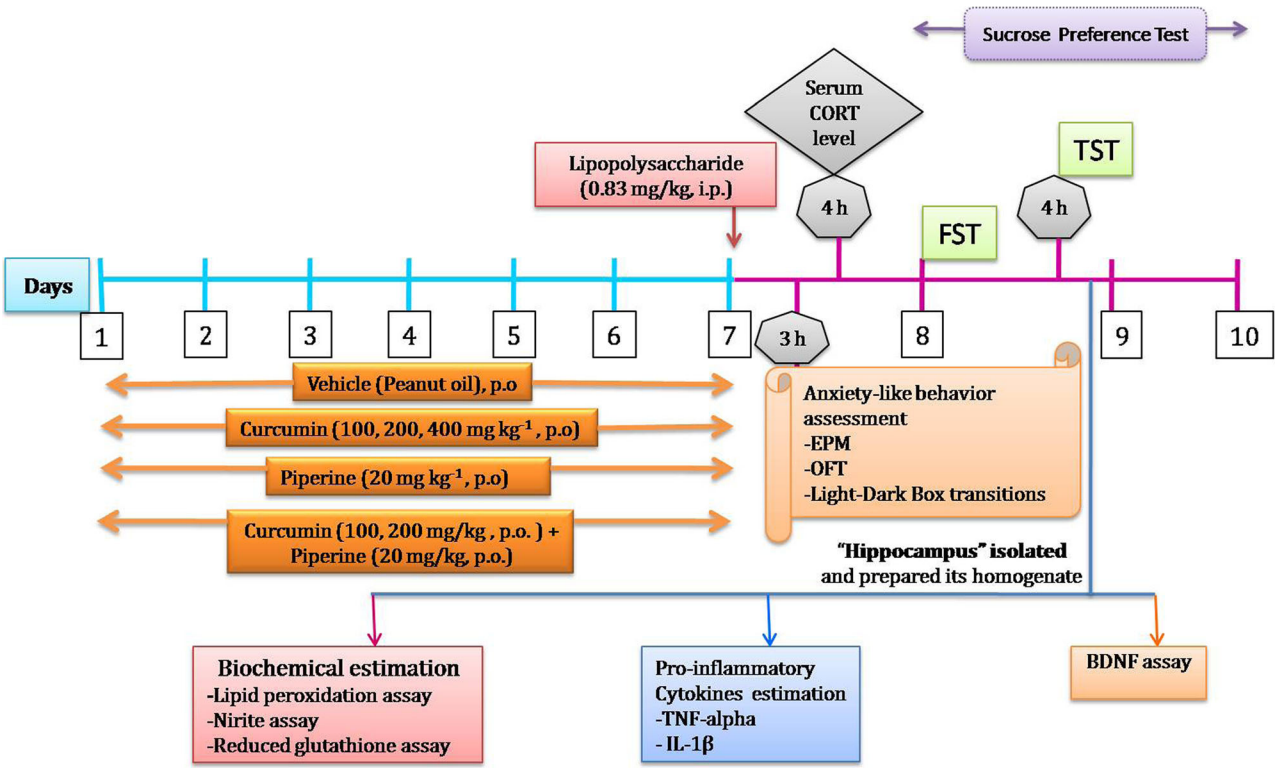


Fig. 2. Illustration of experimental timeline and study plan. Peanut oil (vehicle), curcumin (100, 200, 400 mg/kg), piperine (20 mg/kg), and drug combined treatment was continued for 7 days. On the 7th day, 30 min after the vehicle and drug treatment, LPS (0.83 mg/kg, i.p.) was administered. After 3 h of LPS challenge, anxiety-like behavior was evaluated by performing elevated plus maze test (EPM), open field test (OFT), and light–dark box test. After 4 h of LPS injection, serum corticosterone was measured. After 24 h of LPS administration, depressive-like behavior was evaluated by performing tail suspension test (TST) and forced swimming test (FST). All the biochemical analyses were done after 24 h of LPS administration. Moreover, the anhedonic response was examined for 24 h, by performing sucrose preference test after 24 h of LPS administration.

Mice were treated with drugs for consecutive 7 days followed by challenged with either saline or LPS (0.83 mg/kg, i.p.). Anxiety-like behavior was evaluated by elevated plus maze (EPM) test, light–dark box test, and open field test (OFT) 3 h after the LPS or saline administration. Depressive-like behavior was assessed by forced swimming test (FST) and tail suspension test (TST) after 24 and 28 h, respectively, after the LPS or saline challenge. All the behavioral parameters were evaluated in a dimly lit, isolated room and recorded with a video camera. Neurobehavioral and neurochemical assessments were performed on different animal groups to avoid the effect of behavioral paradigms on biochemical parameters. The animals used for the neurochemical estimation were sacrificed by cervical dislocation after 24 h of saline or LPS challenge. The hippocampus was isolated quickly and stored at −80 °C until further analysis.

Behavioral Parameters Assessment

Elevated Plus Maze Test

Elevated plus maze (EPM) was used to assess the influence of LPS on the anxiety behavior in mice. It was made of two open arms (35×5 cm^2) perpendicular to the two closed arms of the same size with a small central square (5×5 cm^2) between arms. EPM was elevated 50 cm above the floor in a dimly lit room. Each mouse was placed at the center of elevated plus maze with the head facing toward an open arm and allowed to explore the EPM for 5 min. The number of entries into the open arm and closed arm, percentage open arm entries, percentage time spent in the open arm entries, and end-arm explorations during the test was evaluated and presented [32].

Open Field Test

Open field test (OFT) is a useful tool to evaluate the effect of LPS on the motor and behavioral changes in the mice. It was made of the acrylic black box ($72 \times 72 \times 36$ cm^3) with its floor divided into 16 equal sized squares (18×18 cm^2). Four squares were considered as the centre, and 12 squares along the walls were considered as the periphery. Each mouse was placed in the centre of the box. The number of central and peripheral crossings and rearing movements of mice was observed for 10 min by a video camera [33].

Light–Dark Box Test

This test was performed to assess the anxiety behavior in the rodents. The light–dark box comprised of two different compartments: a light chamber ($42 \times 30 \times 20$ cm^3; white walls and brightly illuminated with 40 W bulb) and a dark chamber ($42 \times 30 \times 20$ cm^3; opaque black walls and dark), with an opening (6×6 cm^2) between the two compartments and a video camera located 50 cm above the box. Individual mouse was kept in a dark side with head facing towards the illuminated side and allowed to explore for 10 min. Percentage time spent in the light compartment, the number of light–dark transitions, and percentage time of risk assessments were evaluated and presented [34].

Sucrose Preference Test

Sucrose preference test was performed to examine the anhedonic response after LPS administration. We employed two-bottle paradigm (one with 2 % sucrose solution and other one having plain drinking water) from which mice could select one between two bottles. A week before the initiation of the experiment, all mice were incorporated in this test for 24 h period to determine the baseline consumption and to minimize the response to novelty. Water and food were given to the animals *ad libitum* before and during the test. Sucrose preference test was performed after 24 h of saline or LPS administration by keeping the bottles containing drinking water and sucrose solution for the next 24 h. The bottles were interchanged after every 6 h to avoid side preference. Sucrose consumption was calculated via using an equation: % Sucrose consumption = sucrose intake/total fluid intake (water + sucrose intake) 100 (Sulakhiya et al. 2014).

Tail Suspension Test

Tail suspension test (TST) was performed according to the method [35]. Mice were suspended 40 cm above the floor by an adhesive tape placed approximately 1 cm from the tip of the tail. Mice were suspended for 6 min, and the immobility time during the last 5 min period was analyzed and presented in seconds.

Forced Swimming Test

Forced swimming test (FST) was performed as per the following standard protocol with minor modification [36]. Mice were separately forced to swim in an open cylindrical container (10 cm diameter, 25 cm height), containing water (25 ± 1 °C) to a depth of 20 cm. The immobility time was defined as the absence of escape-oriented

behaviors. Each animal was forced to swim for 6 min, and the total time of immobility in seconds was calculated during last 5 min.

Estimation of Oxidative Stress Parameters

Lipid peroxidation and reduced glutathione levels were estimated to assess the oxidative stress in the hippocampus region. The thiobarbituric acid reactive substances (TBARS) level was quantified according to the method as described earlier [37]. Absorbance for the TBARS level was measured at 532 nm and expressed as μmol of malondialdehyde (MDA)/mg of protein. Reduced glutathione level was estimated by employing the method of Beutler [38]. The results were shown as μg/g of tissue. Protein level was measured according to the Lowry method [39].

Estimation of Nitrite Level

Nitrite estimation was performed in the hippocampus as described earlier [40]. One hundred microliters of Griess reagent was incubated with 100 μl of supernatant of hippocampus homogenate for 10 min at room temperature. Thereafter, absorbance was measured at 560 nm in a microplate reader. Nitrite content was determined from a standard nitrite curve generated by using sodium nitrite as standard. Results were expressed as μM/mg of tissue.

Estimation of Cytokines, Corticosterone, and BDNF Level

IL-1β and TNF-α levels were determined in the hippocampus by using ELISA kits purchased (RayBiotech, Inc). The concentration of the cytokines in 100 μl samples was determined according to the manufacturer's protocol, and the sample values were then read from the standard curve. The concentrations of IL-1β and TNF-α were expressed as pg/ml. Serum corticosterone (CORT) level was determined by corticosterone assay ELISA kit (Abnova Corporation, Taiwan). CORT concentration was measured according to the manufacturer's protocol and expressed as ng/ml. Quantitative determination of BDNF level in the hippocampus was performed by using BDNF assay kit (Promega, Madison, WI, USA). The concentration of BDNF was expressed as ng/mg of protein. All samples and standards were assayed in duplicate.

Statistical Analysis

Experimental data was expressed as the mean ± standard error of mean (SEM). Data were analyzed by one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test by using GraphPad prism 5. $p < 0.05$ was considered significant.

RESULTS

Effect of Curcumin, Piperine, and Their Combination on LPS-Induced Anxiety-Like Behavior

After 3 h of LPS injection, mice showed anxiety-like behavior as evident by the EPM test, open field test, and light–dark box test results (Tables 1, 2, and 3). In EPM test, LPS administration significantly decreased the number of open and closed arm entries ($p < 0.001$ and $p < 0.05$ respectively), % open arm entries ($p < 0.001$), % time spent in open arm ($p < 0.001$), and end-arm explorations ($p < 0.001$) as compared with vehicle control group (Table 1). However, curcumin (200 and 400 mg/kg) pretreatment for 7 days prevented the development of anxiety-like behavior as evident by significantly increased in number of entries in the open arm ($p < 0.05$ and $p < 0.001$ respectively), % open arm entries ($p < 0.001$, $p < 0.001$ respectively), and % time spent ($p < 0.001$, $p < 0.001$ respectively) in the open arm as compared to LPS treated group. Moreover, co-administration of curcumin (100 and 200 mg/kg) with piperine (20 mg/kg) significantly increased the number of entries in the open arm, % open arm entries and % time spent in the open arm when compared with LPS treated group. Furthermore, co-administration of curcumin (200 mg/kg) with piperine (20 mg/kg) significantly increased the number the closed arm entries ($p < 0.05$) and end-arm explorations ($p < 0.001$) as compared to LPS-treated group. In addition, statistical analysis revealed that curcumin (100 and 200 mg/kg) with piperine (20 mg/kg) groups showed significantly increased % time spent in the open arm as compared to curcumin (100 or 200 mg/kg) treatment groups alone.

In OFT, LPS administration significantly reduced the peripheral crossings ($p < 0.001$), central crossings ($p < 0.001$), and rearings ($p < 0.001$) (Table 2). Pretreatment with curcumin (200 and 400 mg/kg) alone significantly protected against LPS-induced reduction in the peripheral crossings ($p < 0.05$, $p < 0.01$ respectively) as well as central crossings ($p < 0.001$, $p < 0.001$ respectively) as compared to LPS-treated group. Curcumin (400 mg/kg) treatment significantly ($p < 0.01$) prevented the reduction in rearings in LPS-treated group. Co-administration of curcumin (100 mg/kg) with piperine (20 mg/kg) significantly prevented LPS-induced reduction in the central crossings ($p < 0.001$) and rearings ($p < 0.05$) as compared to LPS-

Table 1. Effect of Curcumin, Piperine, and Combination Pretreatment on LPS-Induced Changes on the Exploratory Behavior of Mice in the Elevated Plus Maze Test

	No. of entries in closed arm	No. of entries in open arm	% Open arm entries	% Time spent in open arm	End-arm explorations
Vehicle control	9.83 ± 0.83	7.50 ± 0.56	53.39 ± 1.19	36.96 ± 1.17	5.83 ± 0.31
LPS-control	6.33 ± 0.61^{a}	3.83 ± 0.48$^{a'}$	30.78 ± 1.52$^{a'}$	14.76 ± 1.06$^{a'}$	2.5 ± 0.42$^{a'}$
Cur (100 mg/kg) + LPS	6.66 ± 0.67	5.00 ± 0.37	38.11 ± 1.92	20.33 ± 1.05	2.83 ± 0.48
Cur (200 mg/kg) + LPS	7.00 ± 0.37	6.00 ± 0.37^{b}	44.30 ± 1.15$^{b''}$	27.75 ± 1.08$^{b''}$	3.50 ± 0.22
Cur (400 mg/kg) + LPS	8.67 ± 0.42	7.00 ± 0.37^{b} "	48.85 ± 2.72$^{b''}$	30.58 ± 1.15$^{b''}$	4.00 ± 0.37
Pip (20 mg/kg) + LPS	7.33 ± 1.09	5.17 ± 0.31	39.03 ± 2.71	18.58 ± 1.25	2.67 ± 0.42
Cur (100 mg/kg) + Pip (20 mg/kg) + LPS	9.5 ± 0.43	6.50 ± 0.43$^{b'}$	44.85 ± 1.51$^{b''}$	29.85 ± 1.55$^{b'',c'}$	4.33 ± 0.42
Cur (200 mg/kg) + Pip (20 mg/kg) + LPS	10.17 ± 0.74^{b}	7.00 ± 0.37$^{b''}$	50.19 ± 2.18$^{b''}$	33.93 ± 1.59$^{b'',c}$	5.33 ± 0.56$^{b''}$

Results were expressed as the mean ± SEM ($n = 6$ mice/group)
^{a} $p < 0.05$, $^{a'}$ $p < 0.001$ compared with normal control group; ^{b} $p < 0.05$, $^{b'}$ $p < 0.01$, $^{b''}$ $p < 0.001$ compared with LPS control group; ^{c} $p < 0.05$ compared with Curcumin (200 mg/kg); $^{c'}$ $p < 0.001$ compared with Curcumin (100 mg/kg) group

treated group. Furthermore, pretreatment of curcumin (200 mg/kg) in combination with piperine (20 mg/kg) significantly increased rearings when compared to their effects alone.

Table 3 showed that LPS administration significantly decreased the % time spent in light compartment ($p < 0.001$), light–dark transitions ($p < 0.001$), and increased the % time of risk assessment ($p < 0.001$) when compared with vehicle control animals in the light–dark box test. Curcumin (200 and 400 mg/kg) pretreatment significantly prevented the reduced % time spent in light compartment ($p < 0.05$ and $p < 0.001$ respectively) and increased % time of risk assessment ($p < 0.01$, $p < 0.001$) in LPS-challenged animals. Moreover, curcumin (400 mg/kg) alone significantly ($p < 0.01$) prevented the reduction in the light–dark transitions when compared with LPS-treated group. Pretreatment of curcumin (100 and 200 mg/kg) in combination with piperine (20 mg/kg) significantly prevented the LPS-induced alterations in % time spent in light compartment, light–dark transitions, and % time of risk assessment as compared to LPS-treated group. Furthermore, we found that curcumin (100 and 200 mg/kg) and piperine (20 mg/kg) together significantly prevents LPS-induced reduced light–dark transitions when compared to their effects alone. Curcumin (200 mg/kg only) with piperine (20 mg/kg) treatment significantly reduced the % time of risk assessment ($p < 0.001$) in LPS-challenged group when compared to curcumin (200 mg/kg) pretreatment group alone.

Effect of Curcumin, Piperine, and Their Combination on Sucrose Preference Test

Sucrose preference test revealed that LPS administration caused significant ($p < 0.001$) reduction in the sucrose preference (%) index as compared with the normal vehicle control group (Fig. 3). Curcumin administration significantly prevented the LPS-induced anhedonic behavior in

Table 2. Effect of Curcumin, Piperine, and Combination Pretreatment on LPS-Induced Changes on the Exploratory Behavior of Mice in the Open Field Test

	Peripheral crossings	Central crossings	Rearings
Vehicle control	49.33 ± 3.64	24.67 ± 1.26	15.67 ± 0.88
LPS-control	27.83 ± 3.55^{a}	11.67 ± 0.84^{a}	9.33 ± 0.76^{a}
Cur (100 mg/kg) + LPS	39.67 ± 3.04	16.17 ± 1.01	11.50 ± 1.03
Cur (200 mg/kg) + LPS	42.83 ± 2.09^{b}	20.00 ± 1.07$^{b''}$	12.00 ± 1.03
Cur (400 mg/kg) + LPS	45.33 ± 2.01$^{b'}$	22.50 ± 1.18$^{b''}$	14.50 ± 0.76$^{b'}$
Pip (20 mg/kg) + LPS	33.33 ± 2.20	12.83 ± 1.35	12.17 ± 0.75
Cur (100 mg/kg) + Pip (20 mg/kg) + LPS	40.00 ± 3.31	19.50 ± 0.76$^{b''}$	13.33 ± 0.76^{b}
Cur (200 mg/kg) + Pip (20 mg/kg) + LPS	48.50 ± 3.85$^{b''}$	22.00 ± 1.46$^{b''}$	17.00 ± 0.86$^{b'', c}$

Results were expressed as the mean ± SEM (n = 6 mice/group)
^{a} $p < 0.001$ compared with normal control group; ^{b} $p < 0.05$, $^{b'}$ $p < 0.01$, $^{b''}$ $p < 0.001$ compared with LPS control group; ^{c} $p < 0.01$ compared with Curcumin (200 mg/kg) group

Table 3. Effect of Curcumin, Piperine, and Combination Pretreatment on LPS-Induced Changes on the Exploratory Behavior of Mice in the Light–Dark Transition Test

	% Time spent in light compartment	Light–dark transitions	% Time of risk assessment
Vehicle control	36.00 ± 3.14	23.17 ± 1.91	4.25 ± 0.38
LPS-control	18.17 ± 1.74[a]	12.50 ± 0.76[a]	13.17 ± 0.42[a]
Cur (100 mg/kg) + LPS	20.50 ± 1.23	13.83 ± 0.95	11.50 ± 0.62
Cur (200 mg/kg) + LPS	27.33 ± 1.61[b]	15.50 ± 0.62	10.50 ± 0.48[b']
Cur (400 mg/kg) + LPS	30.83 ± 1.90[b"]	18.83 ± 0.95[b']	9.17 ± 0.60[b"]
Pip (20 mg/kg) + LPS	25.00 ± 0.82	14.50 ± 0.43	11.58 ± 0.47
Cur (100 mg/kg) + Pip (20 mg/kg) + LPS	27.83 ± 1.53[b']	20.50 ± 0.76[b",c]	9.75 ± 0.38[b]
Cur (200 mg/kg) + Pip (20 mg/kg) + LPS	34.83 ± 1.30[b"]	22.67 ± 0.49[b",c']	7.25 ± 0.42[b,c']

Results were expressed as the mean ± SEM ($n = 6$ mice/group)
[a] $p < 0.001$ compared with normal control group; [b] $p < 0.05$, [b'] $p < 0.01$, [b"] $p < 0.001$ compared with LPS control group; [c] $p < 0.01$ compared with Curcumin (100 mg/kg) group; [c'] $p < 0.001$ compared with Curcumin (200 mg/kg) group

a dose-dependent manner. Moreover, curcumin (100 and 200 mg/kg) pretreatment in combination with the piperine (20 mg/kg) significantly prevented the LPS-induced anhedonic behavior as compared to LPS treated group as well as curcumin (100 and 200 mg/kg) treatment groups alone.

Effect of Curcumin, Piperine, and Their Combination on LPS-Induced Depressive-Like Behavior

After 24 h of LPS administration, depressive-like behavior was assessed by performing TST and FST. LPS-challenged mice showed significant ($p < 0.001$) increase in the immobility time as compared to vehicle control group in both TST and FST (Fig. 4a, b). This signifies the depressive behavior in LPS-challenged mice. Pretreatment with curcumin (200 and 400 mg/kg) alone significantly prevented the elevation of immobility time in TST and FST. Moreover, we found that curcumin (100 and 200 mg/kg) in combination with piperine (20 mg/kg) showed a significant reduction in immobility time when compared with the LPS-treated group and curcumin (100 and 200 mg/kg) treatment groups alone in both TST and FST.

Effect of Curcumin, Piperine, and Their Combination on Oxido-nitrosative Stress Biomarkers

Figure 5a–c showed that LPS treatment significantly ($p < 0.001$) increased the level of MDA, and nitrite as well as decreased the level of reduced glutathione in the mice hippocampus as compared to vehicle control group. However, curcumin (100, 200, and 400 mg/kg) pretreatment significantly ($p < 0.05$, $p < 0.001$, $p < 0.001$, respectively) prevented the elevation of MDA level in a dose-dependent manner as compared to LPS control group. In addition, curcumin (200 and 400 mg/kg) significantly prevented the LPS-induced elevation in nitrite level and reduction in GSH level as compared to LPS-treated group. Furthermore, co-administration of curcumin (100 and 200 mg/kg) with piperine (20 mg/kg) showed a significant protection against LPS-induced oxido-nitrosative stress in the hippocampus region when compared with LPS control group and curcumin (100 and 200 mg/kg) treatment groups alone.

Effect of Curcumin, Piperine, and Their Combination on Pro-inflammatory Cytokines (IL-1β, TNF-α)

After 24 h of LPS administration, the IL-1β and TNF-α level significantly ($p < 0.001$) increased in the mice

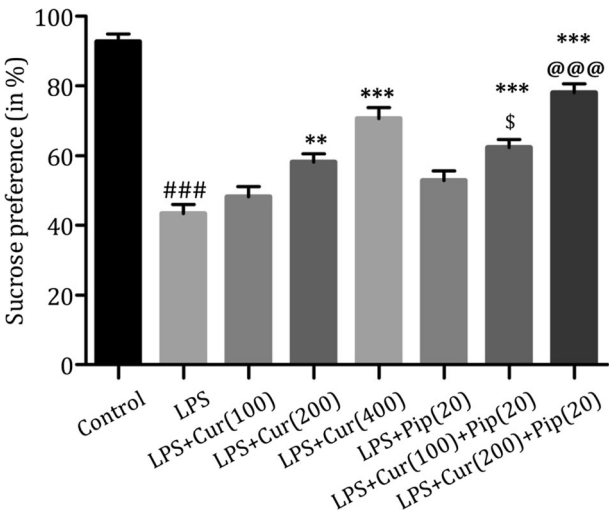


Fig. 3. Effects of curcumin, piperine and combination on sucrose preference test. Results were expressed as mean ± SEM. ($n = 6$). [$] $p < 0.05$; [**] $p < 0.01$; [###, ***, @@@] $p < 0.001$. # *vs* normal control; * *vs* LPS control; $ *vs* curcumin (100 mg/kg) group; @ *vs* curcumin (200 mg/kg) group.

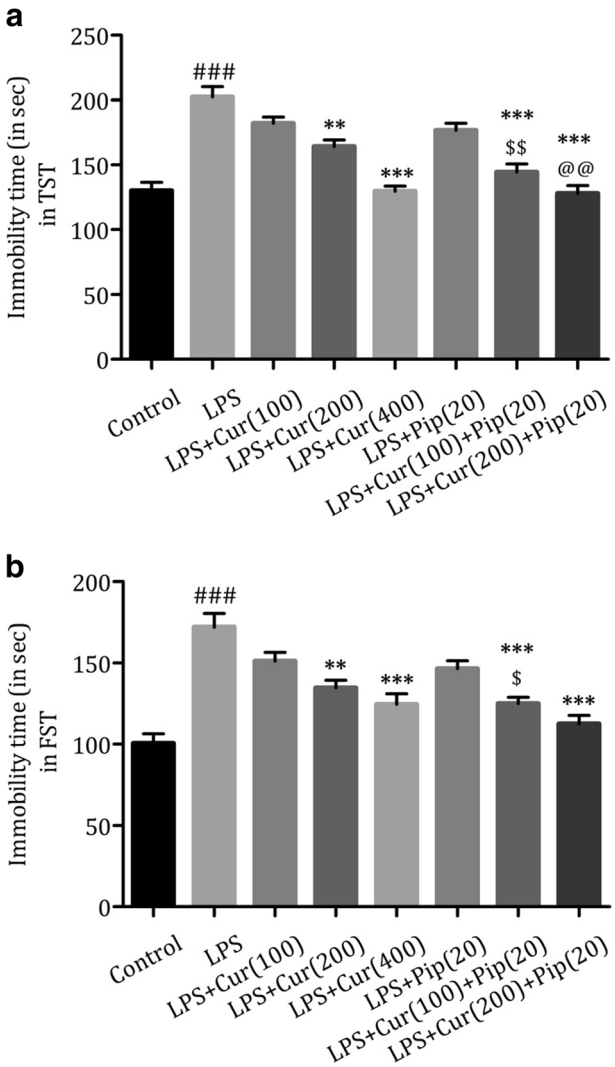


Fig. 4. Effects of curcumin, piperine, and combination pretreatment on LPS-induced changes in **a** Tail suspension test and **b** Forced swim test. Results were expressed as mean ± SEM. ($n = 6$), $^{\$}$ $p < 0.05$; $^{\$\$, **, @@}$ $p < 0.01$; $^{\#\#\#, ***}$ $p < 0.001$. # *vs* normal control; * *vs* LPS control; $ *vs* curcumin (100 mg/kg) group; @ *vs* curcumin (200 mg/kg) group.

hippocampus as compared to vehicle control group (Fig. 6a, b). Curcumin (400 mg/kg) significantly ($p < 0.001$) prevents the augmentation of IL-1β level in the LPS-challenged mice. Curcumin (100 mg/kg) alone treatment did not show significant prevention of LPS-induced increase in IL-1β level. Furthermore, curcumin (200 and 400 mg/kg) treatment groups showed significant ($p < 0.01$, $p < 0.001$, respectively) low level of TNF-α when compared with LPS control group. Co-administration of curcumin (100 and 200 mg/kg) with

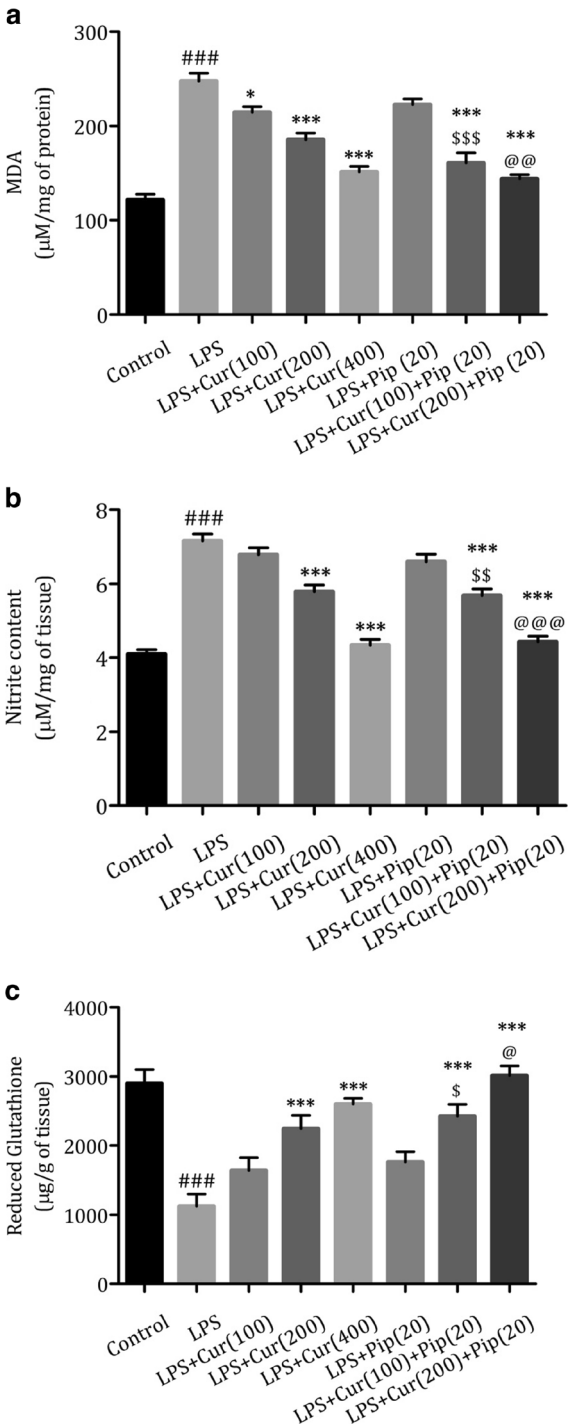


Fig. 5. Effect of curcumin, piperine, and combination pretreatment on LPS-induced changes in **a** MDA level, **b** reduced glutathione level, and **c** nitrite content in the hippocampus. Results were expressed as mean ± SEM ($n = 6$). $^{\$, *, @}$ $p < 0.05$; $^{\$\$, @@}$ $p < 0.01$; $^{\$\$\$, \#\#\#, ***, @@@}$ $p < 0.001$. # *vs* normal control; * *vs* LPS control; $ *vs* curcumin (100 mg/kg) group; @ *vs* curcumin (200 mg/kg) group.

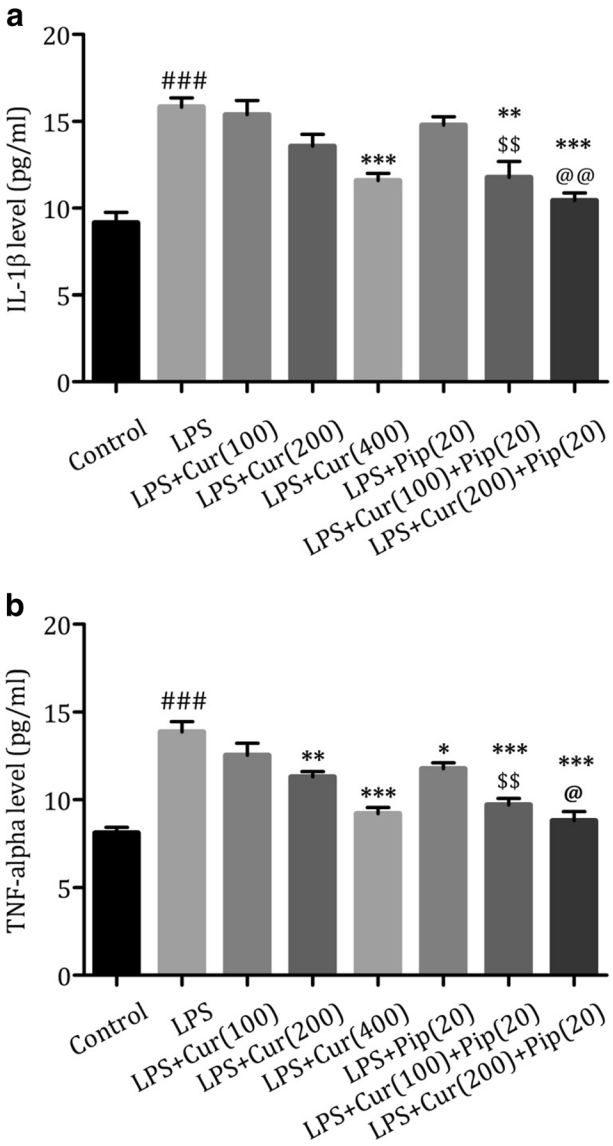


Fig. 6. Effect of curcumin, piperine, and combination pretreatment on LPS-induced changes in **a** tumor necrosis factor-α and **b** interleukin-1β. Results were expressed as mean ± SEM. ($n = 6$). $^{*, @}$ $p < 0.05$; $^{**, \$\$, @@}$ $p < 0.01$; $^{\#\#\#, ***}$ $p < 0.001$. # *vs* normal control; * *vs* LPS control; $ *vs* curcumin (100 mg/kg) group; @ *vs* curcumin (200 mg/kg) group.

piperine (20 mg/kg) potentiated the attenuation effect on pro-inflammatory cytokines (IL-1β and TNF-α) level which was found significant as compared to their effects alone. In addition, piperine (20 mg/kg) alone also significantly prevented the raised level of TNF-α in the hippocampus of LPS challenged group.

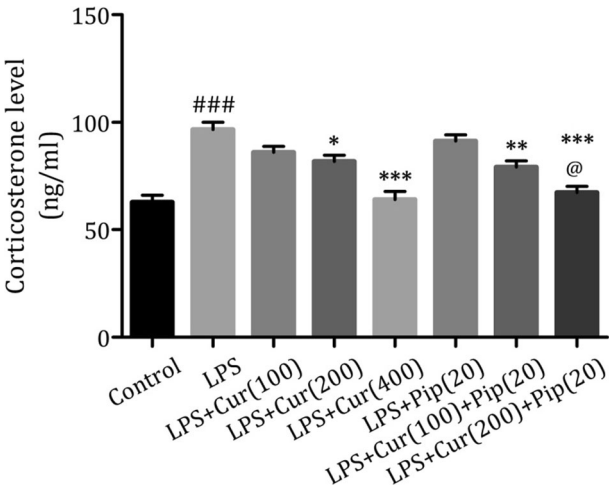


Fig. 7. Effect of curcumin, piperine, and combination pretreatment on LPS-induced changes in plasma corticosterone (CORT) level. Results were expressed as mean ± SEM ($n = 6$). $^{*, @}$ $p < 0.05$; ** $p < 0.01$; $^{\#\#\#, ***}$ $p < 0.001$. # *vs* normal control; * *vs* LPS control; @ *vs* curcumin (200 mg/kg) group.

Effect of Curcumin, Piperine, and Their Combination on Serum Corticosterone and BDNF Level

Figure 7 showed that serum corticosterone level was significantly ($p < 0.001$) increased after 4 h of LPS administration. However, curcumin (200 and 400 mg/kg) pretreatment dose dependently ($p < 0.05$, $p < 0.001$,

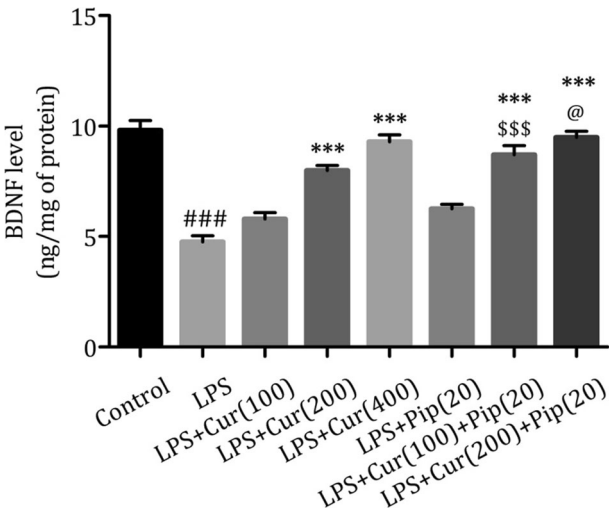


Fig. 8. Effect of curcumin, piperine, and combination pretreatment on LPS-induced changes on BDNF level in the hippocampus. Results are expressed as mean ± SEM ($n = 6$). $^{@}$ $p < 0.05$; $^{\$\$\$, \#\#\#, ***}$ $p < 0.001$. # *vs* normal control; * *vs* LPS control; $ *vs* curcumin (100 mg/kg) group; @ *vs* curcumin (200 mg/kg) group.

respectively) prevented the elevation of corticosterone level in the LPS-challenged group. Furthermore, we found that co-administration of curcumin (100 and 200 mg/kg) in combination with piperine (20 mg/kg) showed the low level of corticosterone which was found significant when compared with LPS-treated group. Moreover, effect of pretreatment of curcumin (200 mg/kg) with piperine (20 mg/kg) on serum corticosterone level was found significant ($p < 0.05$) as compared to their effect alone.

BDNF level was found significantly ($p < 0.001$) reduced in the hippocampus of LPS-challenged mice when compared with vehicle control group (Fig. 8). Pretreatment with curcumin (200 and 400 mg/kg) significantly ($p < 0.001$) prevented the LPS-induced BDNF depletion as compared to the LPS control group. However, curcumin (100 mg/kg) pretreatment did not show a significant effect on BDNF level in the hippocampus when compared with LPS-treated group. However, combination of curcumin (100 and 200 mg/kg) with piperine (20 mg/kg) showed a significant potentiation of their protective effect, i.e., restored BDNF level in the hippocampus.

DISCUSSION

Depression is one of the fatal neuropsychiatric illnesses having lifetime prevalence. There are several interactive pathways involved in the pathogenesis of depression. Moreover, depression is linked with inflammation evidently manifested by raised levels of chemokines, proinflammatory cytokines, and adhesion molecules in the central and peripheral nervous system [41–44]. Anxiety illness is often found in the depression patients [45]. Several studies have revealed the involvement of oxido-nitrosative stress and neuroinflammation in the LPS-induced model of anxiety and depressive-like behavior in rodents [1, 46–48]. Acute administration of LPS causes activation of microglial cells that activate inflammatory cascade which leads to neuroinflammation [1]. LPS administration caused anxiety-like behavior 3 h after LPS injection in mice as evident by the results of EPM, OFT, and light–dark box transition tests. LPS-challenged mice showed the significant reduction in the number of entries in the open and close arm, percentage open arm entries, percentage time spent in open arm, and end-arm explorations in the EPM test. Similarly, LPS-treated animals showed the significant reduction in the peripheral crossings, central crossings, and rearings. Moreover, significant reductions in the percentage time spent in light compartment, light–dark transitions, and increased in the percentage time of risk assessment were found in the LPS-treated group. Our behavioral experimental results reveal that LPS administration leads to anxiety-like behavior in mice which are well corroborated by precedent studies [1, 33, 46, 47, 49]. Moreover, depressive-like behavior was observed in LPS-treated animals in the present study. LPS administration significantly increases the immobility time and despair behavior in FST and TST. There are numerous reports which depicted the depressive-like behavior after 24 h of LPS administration [1, 48, 50–52]. LPS-induced depressive-like behavior accompanied by elevated oxido-nitrosative stress and pro-inflammatory cytokines (IL-1β, TNF-α) in the hippocampus which eventually activates the hypothalamus-pituitary-adrenal (HPA) axis [53, 54]. We also observed anhedonic behavior in LPS-treated mice as evident by reduced sucrose solution consumption.

Previous studies have demonstrated the role of various phytochemicals in mitigation of LPS-induced behavioral anomalies via inhibition of neuroinflammation and oxido-nitrosative stress [1, 48, 55]. In the present study, we have assessed the curcumin alone and in combination with piperine in LPS model of anxiety and depressive-like illness. We found that curcumin treatment alleviated the LPS-induced anxiety-like behavior in a dose-dependent manner. Results were more promising when piperine was co-administered with curcumin. Moreover, curcumin treatment showed the dose-dependent improvement in sucrose solution preference and, therefore, increased sucrose preference percentage index. Co-administration of curcumin with piperine reduced the anhedonic behavior more effectively as compared to curcumin treatment alone. Combination of curcumin with piperine significantly reduced the immobility time in both TST and FST. Our results showed that antidepressant effect of curcumin was more pronounced when given in the combination with piperine. The aforementioned results were well supported with the previous findings [18, 56, 57]. Curcumin has numerous pharmacological properties; however, it has limited therapeutic usefulness due to its poor oral bioavailability [20, 22, 58–60]. To surmount this problem of poor bioavailability, several approaches have been utilized such as curcumin nanoparticles, curcumin-loaded PLGA nanoparticles, nanospheres, liposome-encapsulated curcumin, *etc.* [61–64]. Certain studies have evidently proved piperine as a bioavailability enhancer of curcumin in some diseased model. Piperine acts by affecting the pharmacokinetics of other drugs such as curcumin, quercetin, carbamazepine, *etc.*, thereby enhancing the bioavailability through various mechanisms which are still elusive [30, 31, 65–67].

However, the anticipated mechanism of piperine may be through inhibition of intestinal, hepatic glucuronidation, inhibition of drug-metabolizing enzymes (CYP3A4), and increased absorption of curcumin [68, 69].

LPS administration in acute dose activated the microglia which further released various chemical mediators such as pro-inflammatory cytokines, chemokines in the central nervous system. The pro-inflammatory cytokines raised the level of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that further leads to mitochondrial complexes dysfunction, calcium ions (Ca^{2+}) dysregulation, inflammation, and reduction in the antioxidant profile that ultimately leads to neurobehavioral alterations [70–72]. Our study found that curcumin acts as a neuroprotective agent as it significantly decreased lipid peroxidation, nitrite content and restored GSH level in LPS-challenged mice. Piperine along with curcumin exhibited additive effects in preventing the oxido-nitrosative stress which is corroborated with an earlier study [31]. We also observed the elevated level of IL-1β and TNF-α in the LPS-treated group. Pretreatment with curcumin dose dependently reduced the elevated level of these cytokines. The anti-inflammatory action of curcumin may be due to attenuation of the active inflammatory NF-κB signaling and thus reduced the expression of pro-inflammatory cytokines, chemokines, adhesion molecules, and enzymes (involved in the inflammatory process). Furthermore, LPS-induced pro-inflammatory cytokines cause behavioral alterations through several pathways [73]. The elevated level of cytokines greatly influences the HPA axis through down-regulation of the glucocorticoid receptors (GRs) [53, 74]. Consequently, it results in impaired glucocorticoid negative feedback system. Moreover, these pro-inflammatory cytokines may cause resistance to cortisol by activating certain transcription factors such as AP-1 and NF-κB. These factors form protein–protein complexes with functionally active GRs [75, 76]. The hyperactivity of HPA axis was further confirmed in the certain postmortem study reports [77, 78]. In the current study, the elevated level of serum CORT was observed after 4 h of LPS administration in mice. However, treatment with curcumin alone and in combination with piperine significantly decreased the level of CORT in the serum.cxx

In the present study, we found the lower level of BDNF in LPS-treated group. The neurotrophin hypothesis suggests that the depleted level of BDNF is responsible for the pathogenesis of depression. BDNF signaling plays a putative role in maintaining the brain neuroplasticity, and reduction of BDNF levels has been observed in depression patients which can be restored by some clinical antidepressants [79–81]. Moreover, the precedent studies in the animal models of depression found lower level of BDNF mRNA and protein which was restored with the chronic antidepressant treatment [82, 83]. Furthermore, decrease in BDNF levels is also found to be associated with increase in CORT levels, kynurenine metabolites, and neuroinflammation [17, 84–87]. Pretreatment with curcumin alone and in combination with piperine restored the BDNF level to the normal value. Our results are well supported by the previous findings [57, 85].

CONCLUSION

In summary, the present study indicates that curcumin alone and in combination with piperine exhibited the neuroprotective effect against LPS-induced neurobehavioral and neurochemical deficits in the hippocampus of mice. Piperine exhibited additive effect with curcumin against LPS-induced neurobehavioral deficits. The neuroprotective effect of curcumin may be due to the inhibition of oxido-nitrosative stress and neuroinflammation which further prevented the hippocampal damage effectively when combined with piperine. Hence, piperine, as bioavailability enhancer, improved curcumin performance. Thus, our present study may give insights for the combination of curcumin and piperine to be a potential therapy for depression and anxiety disorders. Moreover, it can be utilized as an adjuvant therapy with current antidepressant drugs.

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